ElisaRSR™ TPOAb Version 2

Thyroid Peroxidase Autoantibody ELISA Kit - Instructions for use

CE

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INTENDED USE

The RSR Thyroid Peroxidase autoantibody (TPOAb) ELISA kit is intended for use by professional persons only for the quantitative determination of thyroid peroxidase (TPO) antibodies in human serum. Autoimmune destruction of the thyroid is associated with the formation of autoantibodies to thyroid peroxidase. Measurement of these autoantibodies is of considerable value in the diagnosis of autoimmune thyroid disease.

REFERENCES

B Rees Smith
"Thyroid autoantibodies"
Scand J Clin Lab Invest 2001 **61** (suppl 235): 45-52

P Burne et al
"Point-of-care assays for autoantibodies to thyroid peroxidase and to thyroglobulin"
Thyroid 2005 **15**: 1005-1010

ASSAY PRINCIPLE

In RSR's TPOAb Version 2 ELISA kit, autoantibodies in calibrators, controls and patient serum are allowed to interact with TPO coated onto ELISA plate wells. After a 15 minute incubation, the samples are discarded leaving TPOAb bound to the immobilised TPO. In a second incubation step, Protein A-Alkaline Phosphatase conjugate binds to the TPOAb bound to the immobilised TPO. The amount of Protein A-Alkaline Phosphatase bound to the plate is then determined in a third incubation step by the addition of p-nitrophenyl phosphate (pNPP) resulting in the formation of a yellow colour. This reaction is stopped by the addition of stop solution and the absorbance of the yellow reaction mixture is then read at 405nm using an ELISA plate reader. A higher absorbance indicates the presence of TPOAb in the test sample. The measuring interval is 10 – 5000 u/mL (NIBSC 66/387).

STORAGE AND PREPARATION OF SERUM SAMPLES

Sera to be analysed should be assayed soon after separation or stored, preferably in aliquots, at or below –20°C. Duplicate determinations using 50 µL of a 1 in 20 dilution of test sample should be made. Repeated freeze thawing or increases in storage temperature must be avoided. Incorrect storage of serum samples can lead to loss of antibody activity. Do not use lipaemic or haemolysed samples. Citrate and heparin plasma may be used in the assay. Studies in which EDTA plasma samples were spiked with TPOAb positive sera showed that for some spiked samples signals more than 10% higher were observed compared with spiked serum from the same donor. In particular, OD_{405} values for spiked EDTA plasma were 112% - 121% of the OD_{405} values for the corresponding spiked serum for 4 of 19 samples tested. These 4 spiked samples had TPOAb concentrations of 747 – 924 u/mL. The concentration range of all 19 spiked samples tested was 14 – 1031 u/mL.

When required, bring test sera to room temperature and mix gently to ensure homogeneity. Centrifuge serum prior to assay (preferably for 5 min at 10-15,000 g in a microfuge) to remove particulate matter. Please do not omit this centrifugation step if sera are cloudy or contain particulates.

SYMBOLS

Symbol	Meaning
CE	EC Declaration of Conformity
IVD	In Vitro Diagnostic Device
REF	Catalogue Number
LOT	Lot Number
	Consult Instructions
	Manufactured By
Σ	Sufficient for
	Expiry Date
8°C 2°C	Store
CONTROL +	Positive Control

MATERIALS REQUIRED AND NOT SUPPLIED

Pipettes capable of dispensing 50 µL and 100 µL.
Means of measuring out various volumes to dilute reagents and test samples.
Pure water.
ELISA Plate reader suitable for 96 well formats and capable of measuring at 405nm.
ELISA Plate shaker, capable of 500 shakes/min (not an orbital shaker).
ELISA Plate washer.
ELISA Plate cover.
ELISA Plate washing machine.

PREPARATION OF REAGENTS SUPPLIED

Store unopened kits and all components at 2-8°C

A	**TPO Coated Wells** 12 breakapart strips of 8 wells (96 in total) in a frame and sealed in foil bag. Allow to stand at room temperature (20-25 °C) for at least 30 minutes before opening.
	Ensure the wells are fitted firmly into frame provided. After opening return any unused wells to the original foil bag, with desiccant provided, seal with adhesive tape, place in the self-seal plastic bag and store at 2-8°C for up to the shelf life of the kit.
B	**Conjugate** **(Protein A-Alkaline Phosphatase)** 11 mL Ready for use
C	**Liquid Substrate (pNPP)** 11 mL Ready to use
D	**Stop Solution** 10.5 mL Ready for use
E	**Assay Diluent** 125 mL Ready for use
F	**Concentrated Wash Solution** 125 mL Concentrated
	Dilute 1 in 10 with pure water. For example, 100 mL (F) + 900 mL pure water. Store at 2-8°C for up to the shelf life of the kit.
G1-5	**Calibrators** 0, 5, 40, 400, 5000 u/mL (units are NIBSC 66/387) 5 x 1.0 mL Ready for use
H1-2	**Positive Controls I & II** **(see label for control range)** 2 x 0.5 mL Concentrated
	Dilute 1:20 with assay diluent (E). For example, 50 µL (H1-2) + 950 µL (E).

ASSAY PROCEDURE

Allow all reagents to stand at room temperature (20-25°C) for at least 30 minutes. A repeating Eppendorf type pipette is recommended for steps 5, 8 and 9.

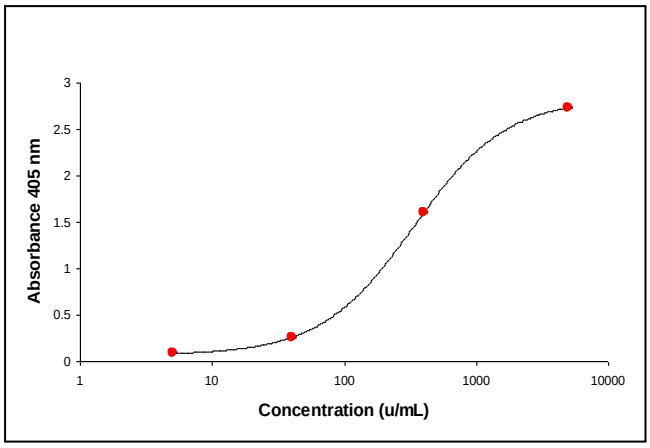
1.	Dilute all serum samples and kit positive controls 1:20 with assay diluent (E) (e.g. 50 µL of serum with 950 µL assay diluent). Do not dilute calibrators.
2.	Pipette 50 µL of diluted patient serum, calibrators (G1-5) and diluted controls (H1-2) into respective wells, leaving one well empty for blank (see step 10).
3.	Cover the frame and shake the plate for 15 minutes at room temperature on an ELISA plate shaker (500 shakes per min.).
4.	Aspirate and wash/aspirate the wells three times with diluted wash solution (F) by use of a plate washing machine.
5.	Pipette **100 µL** of conjugate (B) into each well (except blank). Avoid splashing the material out of the wells during addition.
6.	Cover the frame and shake the plate for 15 minutes at room temperature on an ELISA plate shaker (500 shakes per min.).
7.	Repeat wash step 4.
8.	Pipette **100 µL** of liquid substrate (C) into each well (including blank) and incubate at room temperature in the dark for 15 minutes without shaking.
9.	Pipette **100 µL** stop solution (D) to each well (including blank). Ensure substrate incubations are the same for each well.
10.	Within 30 minutes, read the absorbance of each well at 405nm using an ELISA plate reader blanked against the well containing **100 µL** of substrate (C) and **100 µL** stop solution (D) only.

RESULT ANALYSIS

A calibration curve can be established by plotting calibrator concentration on the x-axis (log scale) against the absorbance of the calibrators on the y-axis (linear scale). The TPOAb concentrations in patient sera can then be read off the calibrator curve (plotted at RSR as a 4-parameter curve). Other data reduction systems can be used. Serum samples of concentration greater than 5000 u/mL can be diluted further using assay diluent to bring them within the measuring interval of the assay.

TYPICAL RESULTS (Example only; not for use in calculation of actual results)

Sample	A405 (minus blank)	u/mL
G1	0.020	0
G2	0.088	5
G3	0.260	40
G4	1.607	400
G5	2.734	5000
Control H1	0.265	40
Control H2	1.156	230



ASSAY CUT OFF

Cut off	u/mL
Negative	< 10
Positive	≥ 10

CLINICAL EVALUATION

Clinical Specificity

Sera from 199 healthy blood donors were tested in the TPOAb ELISA V2 kit. 189 (95%) of the sera were negative for TPOAb

Clinical Sensitivity

Sera from 66 patients diagnosed with Graves' or Hashimoto's disease were tested in the TPOAb ELISA V2 kit. 50 (76%) of the sera were positive for TPOAb

Lower Detection Limit

The 0 u/mL calibrator was assayed 20 times and the mean and standard deviation calculated. The lower detection limit at +2 standard deviations was 1.05 u/mL.

Inter Assay Precision

Sample	Mean u/mL (n = 20)	CV (%)
1	12.3	8.1
2	86	5.4
3	194	6.5

Intra Assay Precision

Sample	Mean u/mL (n = 25)	CV (%)
A	24	6.9
B	78	3.4
C	352	4.8

Clinical Accuracy

Analysis of sera from patients with autoimmune diseases other than Graves' or Hashimoto's disease indicated that 14% (n = 7) of sera positive for antibodies to GAD, 38% (n = 8) of sera positive for antibodies to dsDNA, 36% (n = 11) of sera positive for antibodies to the acetylcholine receptor, 75% (n = 4) of sera positive for antibodies to 21-OH and 3% (n = 30) of sera for Rheumatoid Factor were positive in the ElisaRSR™ TPOAb V2 and RiaRSR™ TPOAb. No sera positive for IA-2 Ab (n = 8) or AQP4 Ab (n = 2) were positive for TPOAb in the ElisaRSR™ TPOAb V2

Interference

No interference was observed when samples were spiked with the following materials; haemoglobin up to 500 mg/dL, bilirubin up to 20 mg/dL or intralipid up to 3000 mg/dL.

The data quoted in these instructions should be used for guidance only. It is recommended that each laboratory include its own panel of control samples in the assay. Each laboratory should establish its own normal and pathological reference ranges for TPOAb levels.

SAFETY CONSIDERATIONS

This kit is intended for *in vitro* use by professional persons only. Follow the instructions carefully. Observe expiry dates stated on the labels and the specified shelf life for coated wells and diluted reagents. Refer to Safety Data Sheet for more detailed safety information. Material of human origin used in the preparation of the kit has been tested and found non-reactive for HIV1 and 2 and HCV antibodies and HBsAg but should none-the-less be handled as potentially infectious. Wash hands thoroughly if contamination has occurred and before leaving the laboratory. Sterilise all potentially contaminated waste, including test specimens before disposal. Material of animal origin used in the preparation of the kit has been obtained from animals certified as healthy but these materials should be handled as potentially infectious. Some components contain small quantities of sodium azide, as preservative. With all kit components, avoid ingestion, inhalation, injection and contact with skin, eyes and clothing, Avoid formation of heavy metal azides in the drainage system by flushing any kit component away with copious amounts of water.

ASSAY PLAN

Allow all reagents and samples to reach room temperature (20-25 °C) before use	
Dilute:	Positive controls and patient samples 1:20 (e.g. 50 µL serum to 950 µL assay diluent). Do not dilute calibrators.
Pipette:	**50 µL** Calibrators, positive controls, patient sera into wells (except blank well)
Incubate:	15 Minutes at room temperature on an ELISA **plate shaker at 500 shakes/min**
Aspirate:	Plate
Wash/Aspirate:	Plate three times
Pipette:	**100 µL** Conjugate into each well (except blank well)
Incubate:	15 Minutes at room temperature on an ELISA **plate shaker at 500 shakes/min**
Aspirate:	Plate
Wash/Aspirate:	Plate three times.
Pipette:	**100 µL** Liquid substrate into each well (including blank well)
Incubate:	15 Minutes at room temperature **in the dark without shaking**
Pipette:	**100 µL** Stop solution into each well (including blank well)
Read absorbance at 405 nm within 30 minutes of adding stop solution	